Data Evaluation Report on the Acute Toxicity of Dithane M-45 to Rainbow Trout (*Oncorhynchus mykiss*)
PMRA Submission Number {.....} EPA MRID Number 45934701

Data Requirement:

PMRA DATA CODE	{.....}
EPA DP Barcode	D293472
OECD Data Point	
EPA MRID	45934701
EPA Guideline	§72-1c

Test material: Dithane M-45 **Purity:** 81.3%
Common name: Mancozeb
Chemical name: IUPAC: Not reported
CAS name: Not reported
CAS No.: 8018-01-7
Synonyms: None specified

Primary Reviewer: Christie E. Padova
Staff Scientist, Dynamac Corporation
Signature: C.E. Padova
Date: 11/7/03

QC Reviewer: Teri Myers, Ph.D.
Staff Scientist, Dynamac Corporation
Signature: Teri Myers
Date: 11/7/03

Primary Reviewer: Gabe Patrick, Biologist
OPP/EFED/ERB - V
Date: G. Patrick 2/6/04

Secondary Reviewer(s):
{EPA/OECD/PMRA}
Date:

Reference/Submission No.:

Company Code:
Active Code:
EPA PC Code: 014504

Date Evaluation Completed:

CITATION: Rhodes, J.E. 2000. Acute Toxicity of Dithane® M-45 to the Rainbow Trout (*Oncorhynchus mykiss*) Determined Under Flow-Through Test Conditions. Unpublished study performed by ABC Laboratories, Inc., Columbia, MO. Laboratory Study No. 46040. Study submitted by Rohm and Haas Company, Spring House, PA. Study initiated May 24, 2000 and submitted October 20, 2000.

2020210

EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, Rainbow trout (*Oncorhynchus mykiss*) were exposed to Dithane M-45 (mancozeb) at nominal concentrations of 0 (negative control), 0.13, 0.25, 0.50, 1.0, and 2.0 ppm under flow-through conditions. Mean-measured concentrations were <0.06 (LOQ, negative control), 0.14, 0.27, 0.45, 1.1, and 1.9 ppm a.i.

After 96 hours of exposure, mortality was 0% at the control, 0.14, and 0.27 ppm a.i. test levels, 5% at the 0.45 ppm a.i. level, 45% at the 1.1 ppm a.i. level, and 100% at the 1.9 ppm a.i. level. The 96-hour LC_{50} (with 95% C.I.) was 0.91 (0.77-1.1) ppm a.i., which categorizes Dithane M-45 as highly toxic to Rainbow trout (*Oncorhynchus mykiss*) on an acute toxicity basis. Exopthalmia, loss of equilibrium, and/or resting on the bottom of the test chamber were observed in surviving fish from treatment groups ≥0.45 ppm a.i. between 24 and 96 hours. The NOEC and LOEC for mortality and sub-lethal effects were 0.27 and 0.45 ppm a.i., respectively.

The test substance, mancozeb, has poor water solubility. Mancozeb's water solubility is roughly 6 ppm. The EPA's Rejection Rate Analysis (US EPA. December, 1994) determined studies were to use measured as opposed to nominal concentrations to discover aquatic toxicological endpoints for compounds that have poor solubility. Filtering of the test solution before analytical measurement increases the accurate measurement of the test material in solution because this removes the undissolved material in the solution (MRID No. 43525001, Monk, 1994). This remaining, soluble portion of the chemical is more biologically available to aquatic organisms and represents a more conservative estimate of the toxicity to these organisms. This study uses measured but unfiltered samples to decide the toxicological endpoints. (gp)

This study is scientifically sound, but it does not fulfill the guideline requirements for an acute toxicity test with freshwater fish (§72-1c) because terminal wet weights of control fish averaged 0.464 g, which is lower than the minimum required initial weight of test fish (0.5-5 g) and the results are from measured but unfiltered samples. As a result, this study is classified as Supplemental.

Results Synopsis

Test Organism Size/Age (mean Weight or Length): Not specified; 0.464 g and 33 mm (means of control fish at study termination)
Test Type (Flow-through, Static, Static Renewal): Flow-through

96-Hour
NOEC: 0.27 ppm a.i.
LOEC: 0.45 ppm a.i.
LC_{50}: 0.91 ppm a.i. 95% C.I.: 0.77-1.1 ppm a.i.
Probit Slope: N/A

Endpoints affected: Mortality and sub-lethal effects

I. MATERIALS AND METHODS

GUIDELINES FOLLOWED: The study protocol was based on procedures outlined in the U.S. EPA Ecological Effects Test Guidelines, OPPTS 850.1075 (1996); and OECD Guidelines for Testing of Chemicals, Guideline No. 203 (1984). Deviations from U.S. EPA FIFRA Guideline §72-1c included:

1. The initial age, weight, and length of fish were not specified.

2. Terminal wet weights of control fish averaged 0.464 g, which is slightly below the minimum required initial weight of test fish (0.5-5 g).

3. It was not reported if fish were maintained under static or flow-through conditions during acclimation.

4. It was not reported if aeration was employed during the study.

5. The water hardness (142 mg/L as $CaCO_3$) was significantly higher than recommended (40-48 mg/L as $CaCO_3$).

6. The pH range (8.2-8.4) was higher than recommended (7.2-7.6).

7. The concentrations of pesticides, metals, particulate matter, and chlorine in the dilution water were not provided.

Because the average terminal fish weight was lower than the recommended range of initial fish weight, this study is classified as Supplemental.

COMPLIANCE: Signed and dated (2003) GLP, Confidentiality, and Quality Assurance statements were provided. This study was conducted in accordance with the GLP provisions of the U.S. EPA and OECD.

A. MATERIALS:

1. Test Material Dithane® M-45 (mancozeb)

Description: Yellow powdered solid

Lot No./Batch No. : 6229 LKX

Purity: 81.3%

Stability of Compound Under Test Conditions: The stability of the test substance in the dilution water during the course of the study was demonstrated by analytical determination at 0 and 4 Days. Recoveries (all test levels) were 86.6-113% of nominal concentrations at 0 Days and 87.0-124% at 4 Days (reviewer-calculated from data provided in Table 2, p. 20).

Storage conditions of test chemicals: Room temperature or under refrigeration.

OECD requires water solubility, stability in water and light, pK_a, P_{ow}, and vapor pressure of the test compound. The following OECD requirements were reported (p. 36):

Aqueous solubility: Dispersible (from attached MSDS) (gp)

Percent volatility: 1% water (from attached MSDS) (gp)

2. **Test organism**:

Species: Rainbow Trout (*Oncorhynchus mykiss*)

Age at test initiation: Not reported

Weight at test initiation: Not reported; at study termination, blotted control fish averaged 0.464 (0.261-0.720) g.

Length at test initiation: Not reported; at study termination, control fish averaged 88 (29-38) mm.

Source: Trout Lodge, Sumner, WA.

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding Study: A 96-hour static range-finding test was conducted with nominal concentrations of 0 (negative control), 0.38, 0.75, 1.5, 3.0, and 6.0 ppm (p. 12). After 96 hours, mortality was 0% in the control group, 80% in the 0.38 ppm group, and 100% in the ≥0.75 ppm groups.

A second range-finding study was conducted under flow-through conditions at nominal concentrations of 0 (negative control), 0.13, 0.25, 0.50, 1.0, and 2.0 ppm. After 96 hours, mortality was 0% in the control and ≤0.50 ppm groups, 80% in the 1.0 ppm group, and 100% in the 2.0 ppm group.

b. Definitive Study:

Table 1: Experimental Parameters

Parameter	Details	Remarks Criteria
Acclimation period:	14-day holding period, and 48-hour acclimation (to temperature) period.	
Conditions: (same as test or not)	Not specified	
Feeding:	Salmon starter and/or brine shrimp nauplii were provided daily. Food was withheld 48 hours prior to and during exposure.	*EPA requires: minimum 14 days; no feeding during test OECD requires minimum of 12 days.*
Health: (any mortality observed)	0% mortality in the 48 hours prior to testing	
Duration of the test	96 hours	EPA/OECD requires: 96 hours

Parameter	Details	Remarks Criteria
Test condition static/flow through Type of dilution system- for flow through method. Renewal rate for static renewal	 Flow-through Intermittent-flow proportional diluter N/A	The flow rate was equivalent to approximately 10 turnovers/day. The test system was calibrated prior to use, and monitored for proper function twice daily. *EPA: Must provide reproducible supply of toxicant, with a consistent flow rate of 5-10 vol/24 hours, and meter systems calibrated before study and checked twice daily during test period*
Aeration, if any	Not reported.	*EPA requires: no aeration; OECD permits aeration*
Test vessel Material: (glass/stainless steel) Size: Fill volume:	Glass aquaria 23 x 31 x 32 cm (23 L) 15 L (21-cm depth)	*EPA requires: Size 19 L (5 gal) or 30 x 60 x 30 cm* *Fill volume: 15-30 L of solution*
Source of dilution water	The dilution water was moderately hard freshwater prepared by blending naturally hard well water with well water that was de-mineralized by reverse-osmosis (p. 11). The blended water was passed through a sediment filter and UV-sterilized prior to use.	*EPA 1975; Soft reconstituted water or water from a natural source, **not** dechlorinated tap water;* *OECD permits dechlorinated tap water.*

Parameter	Details	Remarks Criteria
Water parameters: Hardness pH Dissolved oxygen Total Organic Carbon Particulate Matter Metals Pesticides Chlorine Temperature Intervals of water quality measurement	 142 mg/L as $CaCO_3$ 8.2-8.4 9.0-9.9 mg/L (85-98% saturation) 0.40 mg/L Not reported Not reported Not reported Not reported 11.0-13.0°C Hardness and TOC were measured in the dilution water prior to the start of the study. Temperature, oxygen content, and pH of the test water were measured in all replicate vessels (with surviving organisms) at 0, 24, 48, 72, and 96 hours. Temperature was also monitored continuously in a centrally-located vessel.	Water hardness greatly exceeded recommendations. The pH range was slightly higher than recommended. ***Hardness and pH*** *EPA requires hardness of 40-48 mg/L as $CaCO_3$ and pH of 7.2-7.6. OECD allows hardness of 10-250 mg/L as $CaCO_3$ and pH between 6 and 8.5.* ***Dissolved Oxygen*** *Renewal: ≥60% during 1st 48 hrs and ≥ 40% during 2nd 48 hrs* *Flow-through: ≥60% through out test. OECD requires at least 80% saturation value.* ***Temperature*** *EPA requires 12 °C for coldwater species and 17-22 °C for warmwater species. OECD requires range of 21 - 25 °C for bluegill and 13-17 °C for rainbow trout.* ***EPA water quality*** *measured at beginning of test and every 48 hours*
Concentration of test material: nominal: measured:	 0 (negative control), 0.13, 0.25, 0.50, 1.0, and 2.0 ppm. <0.06 (LOQ, negative control), 0.14, 0.27, 0.45, 1.1, and 1.9 ppm a.i.	Mean-measured concentrations are provided in Table 2, p. 20. *EPA/OECD requires: Control and five treatment levels. Each conc. should be 60% of the next highest conc., and should be in a geometric series*
Solvent (type, percentage, if used)	None used.	*EPA requires: Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests; OECD requires solvent, exceed 100 mg/L.*

Parameter	Details	Remarks Criteria
Number of fish/replicates: negative control:	20 fish, divided between 2 replicates	
solvent control: treated:	N/A 20 fish/level, divided between 2 replicates/level	*EPA: ≥ 10/concentration; OECD requires at least 7 fish/concentration*
Biomass loading rate	0.31 g/L (instantaneous)	Loading rate was reviewer-calculated: 0.464 g (average fish weight) x 10 fish/vessel ÷ 15 L volume per vessel = 0.31 g/L. *Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day; OECD requires maximum of 1 g fish/L for static and semi-static with higher rates accepted for flow-through*
Lighting	16-hours light/8-hours dark, with a 30-minute dusk/dawn simulation.	*EPA requires: 16 hours light/8 hours dark); OECD requires 12 -16 hours photoperiod.*
Feeding	Animals were not fed during testing.	*EPA/OECD requires: No feeding during the study*
Stability of chemical in the test system	Verified. The measured concentrations ranged from 86.6-113% of nominal concentrations on Day 0 and 87.0-124% on Day 4 (reviewer-calculated from data provided in Table 2, p. 20).	
Recovery of chemical Level of Quantitation Level of Detection	95-125% of nominal 0.0597 ppm a.i. Not reported	Based on quality control (QC) spikes run concurrently with the test samples (Table 2, p. 20).
Positive control {if used, indicate the chemical and concentrations}	None tested	
Other parameters, if any	N/A	

2. Observations:

Table 2: Observations

Criteria	Details	Remarks/Criteria
Parameters measured including the sub-lethal effects/toxicity symptoms	Mortality and sub-lethal effects	
Observation intervals	24, 48, 72, and 96 hours	*EPA/OECD requires: minimally every 24 hours*
Were raw data included?	Yes	
Other observations, if any	N/A	

II. RESULTS AND DISCUSSION:

A. MORTALITY:

After 96 hours of exposure, mortality was 0% at the control, 0.14, and 0.27 ppm a.i. test levels, 5% at the 0.45 ppm a.i. level, 45% at the 1.1 ppm a.i. level, and 100% at the 1.9 ppm a.i. level (Table 3, p. 21). The 96-hour LC_{50} (with 95% C.I.) was 1.0 (0.83-1.2) ppm a.i. The NOEC (for mortality) was 0.27 ppm a.i.

Table 3: Effect of Dithane M-45 on mortality of Rainbow trout (*Oncorhynchus mykiss*).

Treatment, ppm a.i., measured and (nominal conc.)	No. of fish at start of study	Observation Period					
		24 Hours		72 Hours		96 Hours	
		No Dead	% mortality	No Dead	% mortality	No Dead	% mortality
Negative control	20	0	0	0	0	0	0
0.14 (0.13)	20	0	0	0	0	0	0
0.27 (0.25)	20	0	0	0	0	0	0
0.45 (0.50)	20	0	0	1	5	1	5
1.1 (1.0)	20	0	0	2	10	9	45
1.9 (2.0)	20	0	0	13	65	20	100
NOEC (mortality), ppm a.i.		Not reported		Not reported		0.27	
LC_{50} (95% C.I.), ppm a.i.		>1.9		1.7 (1.3-2.6)		1.0 (0.83-1.2)	
Positive control, if used mortality: LC_{50}:		N/A		N/A		N/A	

B. NON-LETHAL TOXICITY ENDPOINTS:

Exopthalmia, loss of equilibrium, and/or resting on the bottom of the test chamber were observed in surviving fish from the ≥0.45 ppm a.i. test levels between 24 and 96 hours (Table 3, p. 21). The NOEC for sub-lethal effects was 0.27 ppm a.i.

Table 4: Sub-lethal effects of Dithane M-45 on Rainbow trout (*Oncorhynchus mykiss*).

Treatment, ppm a.i. Measured and (nominal) concn.	Observation Period			
	endpoint at 24 Hours	endpoint at 48 Hours	endpoint at 72 Hours	endpoint at 96 Hours
	% affected	% affected	% affected	% affected
Negative control	N	N	N	N
0.14 (0.13)	N	N	N	N
0.27 (0.25)	N	N	N	N
0.45 (0.50)	N	N	Exopthalmia and resting on bottom - 36%	Exopthalmia and resting on bottom - 36%
1.1 (1.0)	N	N	Exopthalmia and resting on bottom - 100%	Exopthalmia and resting on bottom - 100%
1.9 (2.0)	Resting on bottom- 5%	Loss of equilibrium - 7%	Exopthalmia and resting on bottom - 100%	—
NOEC, ppm a.i.	0.27			
LOEC, ppm a.i.	0.45			
EC_{50}, ppm a.i.	Not reported			
Positive control, if used % sublethal effect: EC_{50}:	N/A	N/A	N/A	N/A

N - Appeared normal.
— Complete mortality.

C. REPORTED STATISTICS:

The 96-hour LC_{50} value (with 95% C.I.) was calculated using probit analysis (Finney, 1971). The NOEC for mortality and sub-lethal effects was based on visual inspection of the effects data. Mean-measured concentrations were used for the calculations.

96-Hour
NOEC: 0.27 ppm a.i.
LOEC: 0.45 ppm a.i.
LC_{50}: 1.0 ppm a.i. 95% C.I.: 0.83-1.2 ppm a.i.
Probit Slope: 6.2

Endpoints affected: Mortality and sub-lethal effects

D. VERIFICATION OF STATISTICAL RESULTS:

The 96-hour LC_{50} was determined using the moving average method via TOXANAL statistical software; this method provided a slightly narrower 95% confidence interval than the probit method. The NOEC was visually determined. Mean-measured concentrations were used for the calculations.

96-Hour
NOEC: 0.27 ppm a.i.
LOEC: 0.45 ppm a.i.
LC_{50}: 0.91 ppm a.i. 95% C.I.: 0.77-1.1 ppm a.i.
Probit Slope: N/A

Endpoints affected: Mortality and sub-lethal effects

E. STUDY DEFICIENCIES:

Average terminal fish weight was lower than the recommended range of initial fish weight; aside from this deviation from U.S. EPA guideline §72-1c (which impacted the study acceptability), there were no deficiencies in this study.

F. REVIEWER'S COMMENTS:

The reviewer's conclusion was identical to the study author's; Dithane M-45 is categorized as highly toxic to Rainbow trout (*Oncorhynchus mykiss*) on an acute toxicity basis. The reviewer's LC_{50} estimate was slightly lower than the study author's because of the different method used to determine this value. The reviewer's estimate is reported in the Executive Summary and Conclusions sections because it was associated with a slightly narrower 95% confidence interval than the Probit method, which was chosen by the study author.

All test solutions appeared clear, with no visible precipitates or surface film (p. 10). Diluter stock solutions appeared cloudy throughout the exposure period. The test substance, mancozeb, has poor water solubility. Mancozeb's water solubility is roughly 6 ppm. The EPA's Rejection Rate Analysis (US EPA. December, 1994) determined studies were to use measured as opposed to nominal concentrations to discover aquatic toxicological endpoints for compounds that have poor solubility. Filtering of the test solution before analytical measurement increases the accurate measurement of the test material in solution because this removes the undissolved material in the solution (MRID No. 43525001, Monk, 1994). This remaining, soluble portion of the chemical is more biologically available to aquatic organisms and represents a more conservative estimate of the toxicity to these organisms. This study uses measured but unfiltered samples to decide the toxicological endpoints. (gp)

The CAS. No. (8018-01-7) provided in this study (Material Safety Data Sheet, p. 32) differs from that CAS. No. (96-45-7) provided in concurrently-submitted MRIDs 46020901 (acute daphnid) and 46020903 (acute trout).

G. CONCLUSIONS:

This study is scientifically sound, but it does not fulfill the guideline requirements for an acute toxicity test with freshwater fish (§72-1c) because terminal wet weights of control fish averaged 0.464 g, which is lower

than the minimum required initial weight of test fish (0.5-5 g) and the results are from measured but unfiltered samples.. As a result, this study is classified as Supplemental. The LC_{50} (with 95% C.I.) was 0.91 (0.77-1.1) ppm a.i., which categorizes Dithane M-45 as highly toxic to Rainbow trout (*Oncorhynchus mykiss*) on an acute toxicity basis. The NOEC (for mortality and sub-lethal effects) was 0.27 ppm a.i.

96-Hour
NOEC: 0.27 ppm a.i.
LOEC: 0.45 ppm a.i.
LC_{50}: 0.91 ppm a.i. 95% C.I.: 0.77-1.1 ppm a.i.
Probit Slope: N/A

Endpoints affected: Mortality and sub-lethal effects

III. REFERENCES:

U.S. EPA. 1989. Toxic Substances Control Act; Good Laboratory Practice Standards; Final Rule (40 CFR, Part 792). *Federal Register*, Vol. 54(158): 34043-34050.

U.S. EPA. 1989. Pesticide Programs; Good Laboratory Practice Standards; Final Rule (40 CFR, Part 160).

Organization for Economic Cooperation and Development. 1997. OECD Principles of Good Laboratory Practice [C(97) 186/Final].

U.S. EPA. December, 1994. Pesticide Reregistration Rejection Rate Analysis: Ecological Effects. Office of Prevention, Pesticides and Toxic Substances (OPPTS). Washington DC, 20460, US. EPA-738-R-94-0235.

U.S. EPA. 1996. Ecological Effects Test Guidelines. OPPTS 850.1075 Fish Acute Toxicity Test, Freshwater and Marine, Public Draft. Office of Prevention, Pesticides and Toxic Substances, Washington DC, EPA 712-C-96-118.

Organization for Economic Cooperation and Development. 1984. OECD Guidelines for Testing of Chemicals. Fish Acute Toxicity Test, OECD Guideline No. 203.

U.S. EPA. 1997. Code of Federal Regulations. Title 40 - Protection of Environment. Fish Acute Toxicity Test. 40 CFR 797.1400.

U.S. EPA. 1982. Pesticide Assessment Guidelines, Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms. EPA 540/9-82-024.

European Community. 1992. Seventh Amendment to Directive 67/548/EEC relating to the classification, packaging and labeling of dangerous substances.

Finney, D.J. 1971. Probit Analysis. Cambridge University Press.

Hamilton, M.A. *et al.* 1977. Trimmed Spearman-Karber Method for Estimating Lethal Concentrations in Toxicity Bioassays. *Environ. Sci. Techol.* 11(7): 714-719. Correction 12(4): 417. 1978.

MRID No. 43525001. Monk, R. (1994) Acute Toxicity Study on the Rainbow Trout of BAS 222 28 F in a Flow-Through System for 96 Hours: Lab Project Numbers: 94/10920: PCP03089: 94/161. Unpublished study prepared by BASF Aktiengesellschaft. 47 p.

APPENDIX 1. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	4.647325E-02	.9081021	.7702763	1.097095

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	.1716104	1	.3148323

SLOPE = 5.833428
95 PERCENT CONFIDENCE LIMITS = 3.416879 AND 8.249976

LC50 = 1.02166
95 PERCENT CONFIDENCE LIMITS = .821994 AND 1.218754

LC10 = .6188646
95 PERCENT CONFIDENCE LIMITS = .3829448 AND .7798737